

## Role of Comorbidities in Establishment of Pulmonary Mycosis in Chronic Obstructive Pulmonary Disease Patients in and Around Rewa

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### ABSTRACT

**Background:** Viruses and bacterias have been already considered as a major cause of chronic obstructive pulmonary disease (COPD) exacerbations; whereas, the major role of fungal colonization and infection is poorly understood.

**Objective:** Keeping this fact in mind the present study was designed to find out the microbes responsible for acute exacerbation of COPD, which is one the common disorder of chronic lung disease in and around Rewa along with the profile of pulmonary fungal infection among COPD patients with and without comorbidities to determine their prevalence, risk factors, and outcome among those patients.

**Patients and methods:** In this prospective cross-sectional analytic study, different samples (sputum, bronchoalveolar lavage, blood, and others) from 180 COPD patients at risk for pulmonary fungal infection were examined using mycological analysis (direct microscopy with lactophenol cotton blue and culture in SDA media). Broncho alveolar lavage and blood samples were examined using the human 1,3- $\beta$ -d-glucan and galactomannan ELISA tests.

**Results:** The prevalence of pulmonary fungal infection was significantly higher in COPD patients with comorbidities (77.5%) whereas COPD patients without comorbidities (53%) ( $P < 0.001$ ), with a predominance of *Candida* and *Aspergillus* spp. in both groups. Mechanical ventilation, corticosteroid therapy, ICU admission and age were major risk factors for pulmonary fungal infection in COPD patients with comorbidities [ $P = 0.013$ , odds ratio (ODR) = 2.24;  $P = 0.029$ , ODR = 2.02;  $P = 0.024$ , ODR = 1.98; and  $P = 0.036$ , ODR = 2.64; respectively]. COPD patients with comorbidities had significantly higher mortality rate (13.8%) compared with COPD patients without comorbidities (3.6%;  $P < 0.05$ ). Blood galactomannan antigen was positive in 19 (10.5%) COPD patients with comorbidities versus 8 (4.4%) in COPD patients without comorbidities ( $P < 0.05$ ).

**Conclusion:** COPD patients with comorbidities had a higher prevalence of pulmonary fungal infection rate compared with COPD patients without comorbidities. Age, mechanical ventilation, corticosteroid therapy, and ICU admission were independent risk factors for pulmonary fungal infection in COPD patients with comorbidities.

**Keywords:** Chronic Obstructive Pulmonary Disease (COPD), comorbidities, pulmonary fungal infection, Sabouraud's Dextrose Agar (SDA)

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## INTRODUCTION

Comorbidities frequently impact chronic obstructive pulmonary disease (COPD) patients and significantly affect the patients' survival, quality of life, and exacerbation frequency (Putchu et al., 2013). It is believed that chronic inflammatory state in COPD may accelerate the natural history of some comorbidities, and hence COPD is considered as a systemic disorder (Divo et al., 2012). Airways of COPD patients are often colonized with potential pathogenic microorganisms (Sethi & Murphy, 2008) and may lead to increased airway inflammation (O'Donnell et al., 2006). The potential role of fungal colonization and infection in the pathogenesis of COPD is poorly understood as bacteria and viruses were usually considered as the major cause of COPD exacerbations. *Aspergillus* spp. is the most common fungal genus to cause pulmonary-associated fungal infections in COPD patients (Bulpa et al., 2007).

The primary objective of this study was to screen COPD patients with comorbidities and COPD without comorbidities for microbiological and serological pieces of evidence for pulmonary fungal infection to determine the prevalence of pulmonary fungal infection among those patients. The second objective was to identify the risk factor for pulmonary function infection among those patients. The third objective was to investigate the frequency of positive fungal culture and the clinical outcomes among those patients.

## MATERIALS AND METHODS

This prospective cross-sectional analytic study included 180 COPD patients at risk for pulmonary fungal infection who were admitted in the Medicine Department Intensive Care Unit. After meeting the inclusion criteria, informed consent was obtained from all study participants and relatives according to the clinical condition of the patients.

### Criteria for Patients Selection

COPD patients who were eligible for enrolment displayed a combination of the following host factors:

1. A history of pre-existing COPD and immunosuppression from corticosteroids or other underlying conditions (e.g. diabetes, malnutrition, and liver cirrhosis).
2. Clinical signs and/or symptoms suggestive of invasive mycosis.
3. Any of the following symptoms of lower respiratory tract infection: expectoration, dyspnoea, or haemoptysis; pleuritic chest pain etc.
4. Fever persists even after minimum 3 days of appropriate antibiotics, or fever relapsing after 5 days or along with continuation of antibiotics
5. Steroid use: at least 4 mg of methylprednisolone (or equivalent) a day for at least 7 days in the past 3 weeks before admission or during the course of the ICU stay for at least 5 days, or a cumulative dose of at least 250 mg of methylprednisolone (or equivalent) in the past 3 months before enrollment (Dutkiewicz & Hage, 2010); Garnacho-Montero et al., 2013).

Exclusion criteria included patients who received systemic antifungal therapy within 3 days before sample collection and patients who refused to participate in the study.

All patients were subjected to clinical and routine laboratory investigations. Radiology, including plain chest radiograph, and high resolution CT of the chest were performed for patients.

Source of the specimen and method of examination according to the site of the lesion involved the following: direct microscopic examination of sputum samples, bronchoalveolar lavage (BAL) samples, and sterile catheter samples, culture examination of pleural fluid and blood samples (Xavier et al., 2009). Sabouraud's Dextrose agar was routinely used. HiChrome agar was used for the identification of some species of *Candida*. Serologic diagnosis was carried out using the human 1,3- $\beta$ -d-glycosidase (Wright et al., 2011) and human galactomannan (GM) ELISA tests (Pfeiffer et al., 2006) of blood and BAL samples.

## Procedures

Collection of sputum samples from the patients according to universal precautions (Xavier et al., 2009). Sputum samples were collected by instructing the patient to cough as deep as possible to expectorate about 5–10 ml in a sterile container, usually early in the morning.

**Specimen transport/storage:** Each specimen was individually collected in a sealed plastic bag with proper legible labeling in sterile containers and sent to the Microbiology laboratory of S.S. Medical college Rewa affiliated with A.P.S. University, Rewa for mycological examination within 4 h after collection, or if it was stored in the refrigerator at 4°C it was sent within 12–24 h.

## Bronchoalveolar lavage

BAL sample was collected by physicians under complete aseptic conditions according to the BTS guideline (2013) (Du Rand et al., 2013) with the help of a white light flexible bronchoscope BAL fluid was collected in a sterile container and transported immediately to the laboratory. Serum samples were also collected from the same patients and stored at – 20°C for serological analysis.

## Serum

Blood samples were collected according to standard laboratory procedures. Serum samples should not be contaminated with fungal spores and/or bacteria. The samples were transported and stored in sealed tubes (Stynen et al., 1995), unexposed to air.

## Evaluation criteria for the diagnosis of fungal infection

We used the EORTC/MSG diagnostic criteria as the reference standard for case definition of invasive aspergillosis (IA), which were classified as definite, probable, or possible (Pfeiffer et al., 2006).

## Statistical analysis

Data were recorded to statistical package for the social sciences (version 20.0; IBM Inc., Armonk, New York, USA). Data were described as mean±SD or frequencies and percentage depending on whether they were quantitative or qualitative, respectively. The  $\chi^2$ -test and the Fisher exact test was used to compare categorical variables. Comparison of quantitative variables between the study groups was made using the Mann–Whitney *U*-test. Univariate and multivariate logistic regression analysis was performed to test for the effect of all important risk factors on the occurrence of fungal infections. *P* values less than 0.05 were considered significant.

## RESULTS

During the study period, 180 COPD patients at risk for pulmonary fungal infection fulfilled the inclusion criteria. There were 80 (46%) COPD patients with comorbidities and 100(54%) COPD patients without comorbidities. The basic characteristics of COPD patients with comorbidities versus those without comorbidities are shown in [Table 1]. There was a statistically significant difference ( $P < 0.05$ ) as regards the mean age between the two groups; the mean age in COPD patients with comorbidities was  $52.3 \pm 8.6$  versus  $55.5 \pm 6.8$  in COPD patients without comorbidities. There was no statistically significant difference as regards sex between the two groups. There was a statistically significant difference as regards forced expiratory volume in 1 s (FEV1%) predicted between the two groups ( $P < 0.05$ ). COPD patients with comorbidities were associated with lower lung function (FEV1%:  $46.5 \pm 18.6$  vs.  $54.8 \pm 19.9$ % predicted in COPD patients without comorbidities).

**Table 1: Basic characteristics of chronic obstructive pulmonary disease patients with comorbidities versus chronic obstructive pulmonary disease patients without comorbidities**

Variables	COPD with Comorbidities (n=)	COPD without Comorbidities (n=)	<i>P value</i>
Age	52.3±8.6	55.5±6.8	0.036*
Sex			
Male	55(67.2)	72(73)	0.274
Female	28(34.2)	25(27)	
Length of Hospital Stay			
< 1 week	27(33)	44(46.6)	0.084
>1 week	55(68.2)	53(57.1)	
FEV1% Predicted	46.5±18.6	54.8±19.9	0.022*
GOLD class			
I	3(3.7)	0(0)	0.267
II	3(3.7)	1(1.04)	
III	21(25.9)	22(22.9)	
IV	16(19.7)	17(17.7)	

Data expressed as n% or Mean±SD. COPD, Chronic obstructive pulmonary disease; FEV1% Forced Expiratory Volume in 1<sup>0</sup> s; GOLD, Global Initiative for Chronic obstructive pulmonary disease. \*Statistical Significant difference (P<0.05)

Among the different comorbid diseases, the common associations included diabetes mellitus (DM), liver cirrhosis, cardiovascular diseases, malignancy, chronic renal failure, and anemia. Diabetes was the most predominant comorbid disease and was recorded in 48 (28.0%) of 180 COPD patients,

followed by liver cirrhosis and cardiovascular diseases in 38 (21.3%) and 23 (12.3%) patients, respectively. Malignancy was present in 8% of cases, including bronchogenic carcinoma in 8.8% and one case of lymphoma [Table 2].

**Table 2: Comorbid diseases associated with chronic obstructive pulmonary disease in the study group**

Comorbid diseases	N%
Uncontrolled DM	48(28.0)
Chronic liver failure	6 (2.8)
Malignancy	17(8)
Bronchogenic carcinoma	16(8.8)
Lymphoma	1(0.6)
Cardiovascular diseases	23(12.3)
Coronary heart disease	11(5.9)
Congestive heart failure	12(6.7)
Liver cirrhosis	38(21.3)
Anaemia	11(5.9)

Data expressed as n%, COPD, Chronic obstructive pulmonary disease; DM, Diabetes Mellitus; The same patient had more than one comorbid disease; \* Statistical Significant difference (P<0.05)

The prevalence of pulmonary fungal infection was significantly higher in COPD patients with comorbidities (77.5%) compared with COPD

patients without comorbidities (53%) (P < 0.001) [Table 3].

**Table 3: Fungal culture results in the study group**

Culture Results	COPD with Comorbidities (n=80)	COPD without Comorbidities (n=100)	P Value
Culture Positive	62(77.5)	53(53)	<0.001
Culture Negative	18(22.5)	47(47)	

COPD, Chronic obstructive pulmonary disease;  $\chi^2=11.647$ ; \* Statistical Significant difference ( $P<0.01$ )

In the present study we did not get statistically significant difference as regards fungal species ( $P > 0.05$ ) with predominance of *Candida* and *Aspergillus* spp., in both groups. There was a statistically significant difference as regards blood GM, which was positive in 18 (20.4%)

COPD patients with comorbidities versus seven (9.2%) COPD patients without comorbidities ( $P < 0.05$ ). However, 1,3- $\beta$ -d-glucan and BAL GM showed no statistically significant difference between the groups ( $P > 0.05$ ) [Table 4].

**Table 4: Fungal culture and serology in the study group**

Variables	COPD with Comorbidities (n=80)	COPD without Comorbidities (n=100)	P Value
<b>Fungal sp</b>			
Candida sp.	32(43)	30(55)	0.790
Candida albicans	19(27.4)	22(38.4)	0.342
Aspergillus sp.	20(29.2)	17(30.3)	0.751
Penicillium	3(2.5)	2(1.3)	
Fusarium	0(0)	2(3.5)	
Aspergillus fumigates	6(6.9)	8(12.4)	0.234
Mixed/combined	8(7.1)	0(0)	
<b>Serological Diagnosis</b>			
Positive GM (cutoff index=0.5 $\mu$ g/ml)			
GM(BAL)	20(23.5)	15(15.8)	0.167
GM(Blood)	18(20.4)	9(9.2)	0.013*
Positive 1,3, $\beta$ -D Glucose(cutoff index=10 $\mu$ g/ml)	13(16.6)	11(12.3)	0.278

Data expressed as n%, COPD, Chronic obstructive pulmonary disease; BAL, Bronchoalveolar lavage; GM, Galactomannan ELISA Assay; \* Statistical Significant difference ( $P<0.05$ )

[Table 5] summarizes the evaluation of risk factors for fungal infection using univariate analysis and multivariate analysis in COPD patients with comorbidities versus COPD patients without comorbidities. Mechanical ventilation, corticosteroid therapy, ICU admission, and age were major risk factors for pulmonary fungal infection [ $P = 0.013$ , odds ratio (ODR) = 2.23;  $P = 0.029$ , ODR = 1.99;  $P = 0.024$ , ODR = 1.94; and  $P = 0.036$ , ODR =

0.4; respectively]. Neutropenia was found in only one patient with COPD with comorbidities, and hence it was difficult to evaluate it as a risk factor for fungal infection in this study. Multivariate analysis showed that age, mechanical ventilation, corticosteroids therapy, and ICU admission were independent risk factors associated with pulmonary fungal infection in COPD patients with comorbidities versus COPD patients without comorbidities.

**Table 5: Risk factors of pulmonary fungal infection in chronic obstructive pulmonary disease patients with comorbidities versus chronic obstructive pulmonary disease patients without comorbidities**

Risk factors	COPD with Comorbidities(n=80)	COPD without Comorbidities(n=100)	P Value	ODR(95%CI) Unstandardized	Unstandardized
Age	52.3±8.6	55.5±6.8	0.036*		
< 40 years	7(7.4)	3(3.3)		0.4(0.010-1.66)	0.43(0.12-1.45)
≥40 years	76(93.7)	94(96.8)	0.22		
Sex					
Male	55(67.2)	72(73)	0.274	1.42(0.74-2.72)	1.51(0.68-2.18)
Female	28(34.2)	25(27)			
Antibiotics					
Yes	69(85)	74(77)	0.186	1.65(0.77-3.5)	1.73(0.79-3.11)
No	14(17)	23(25)			
Corticosteroids					
Yes	51(62.3)	44(46.1)	0.029*	1.99(1.09-3.63)	2.02(1.10-2.96)
No	32(39.6)	53(54.4)			
Immunosuppressive					
Yes	15(18.4)	18(20.1)	0.935	0.97(0.44-2.11)	1.03(0.16-2.22)
No	68(84.3)	79(83.7)			
ICU Admission					
Yes	38(46.1)	30(31.5)	0.024*	1.94(1.05-3.60)	1.98(1.15-3.12)
No	45(55.3)	67(70.1)			
Length of hospital stay					
<1 week	27(33)	44(46.6)	0.084	1.72(0.93-3.18)	0.62(0.29-1.16)
>1 week	55(68.2)	53(57.1)			
Mechanical ventilation					
Yes	26(31.4)	17(19.7)	0.013*	2.23(1.09-4.56)	2.24(1.61-3.72)
No	58(70.2)	80(84.1)			
Neutropenia					
Yes	2(1.8)	0(0)	0.441	3.60(0.14-89.49)	3.71(0.61-51.32)
No	78(96.3)	100(100)			

Data expressed as n%, Mean SD; CI, Confident interval; COPD, Chronic obstructive pulmonary disease; ODR, Odds ratio;

<sup>a</sup>Univariate analysis ODR; <sup>b</sup>Multivariate analysis ODR; \* Statistical Significant difference(P<0.05)

## DISCUSSION

This study was designed to assess the profile of fungal infection among COPD patients with and without comorbidities to determine the prevalence, risk factors, and outcome of pulmonary fungal infection among those patients.

As regards comorbid diseases in this study, the common associations included DM, liver cirrhosis, cardiovascular diseases, malignancy, chronic renal failure, and anaemia. Diabetes was the most predominant comorbid disease and was recorded in 28.0% of patients, followed by liver cirrhosis and cardiovascular diseases in 21.3 and 12.3% patients, respectively. However, bronchogenic carcinoma was recorded in 8.8% of cases. The

current study revealed that COPD patients with comorbidities were associated with significantly lower mean age and lower FEV1% predicted compared with COPD patients without comorbidities; however, there was no statistically significant difference as regards sex.

Multiple comorbidities may coexist in individuals with COPD and play an important role in determining clinical outcomes in COPD, even after control of the confounding factors (Putcha et al., 2014; Dal Negro et al., 2015). Although, COPD patients have normal pulmonary defense mechanisms against *Aspergillus* spp., such as the ingestion of conidia (the ends of some hyphae are rounded and could be confused with yeast, but that are instead called conidia or spores) by pulmonary

macrophages and killing of hyphae by neutrophils. However, there many factors that predispose to colonization and infection with *Aspergillus* spp., including structural changes in lung architecture with the formation of bullae, and the common use of long-term steroid treatment (even inhaled steroids) increases host susceptibility by reducing oxidative killing of the organism by pulmonary macrophages and increases its linear growth by 30–40%. Moreover, comorbidity factors such as diabetes, alcoholism, and malnutrition may further enhance the risk for pulmonary fungal infection in COPD patients (Meersseman et al., 2007).

Recent studies reported that COPD may affect the function of other organs, including the heart, vasculature, muscles, liver, gastroenteric apparatus, kidney, and brain; it is frequently associated with various disorders (Názara & Balóira, 2015) and accelerates lung aging (MacNee et al., 2014). Several comorbidities frequently predominate, particularly in elderly patients, but the relationship linking their prevalence to patients' sex and COPD severity is still unclear (Jo et al., 2015; Miravittles et al., 2015).

In the present study, the prevalence of pulmonary fungal infection was significantly higher in COPD patients with comorbidities (77.5%) compared with COPD patients without comorbidities (53.0%). However, there was a predominance of *Candida* and *Aspergillus* spp., in both groups, with no statistically significant difference. There was statistically significant difference as regards blood GM, which was positive in 18 (20.4%) COPD patients with comorbidities versus seven (9.2%) COPD patients without comorbidities. However, 1,3- $\beta$ -d-glucan and BAL GM showed no statistically significant difference between the two groups. In contrast, Tutar et al. (2013) found that GM with median value 0.54 ng/ml was positive in nine patients (81.8%) and 1,3- $\beta$ -d-glucan was examined in five patients and was positive in three (60%) of them.

As regards the outcome, this study demonstrated that COPD patients with comorbidities at risk factors for pulmonary fungal infection in COPD patients with comorbidities versus COPD patients without comorbidities, age, mechanical ventilation therapy, corticosteroid therapy, and ICU admission were major risk factors for pulmonary fungal infection in COPD patients with comorbidities, with a statistically significant difference. However, antibiotic therapy was prominent in both groups, with no statistically significant difference. Similarly, in recent years, it has been shown that corticosteroid use plays a significant role in terms of increasing the rate of invasive pulmonary aspergillosis (IPA) incidence in COPD cases (Guinea et al. 2010). In a retrospective study, it was shown that steroid use of over 700 mg in total within the last three months in COPD patients increased the risk for IPA (Stynen et al. 1995). Moreover, it has been stated by other authors that inhaled steroids are a risk factor for IPA (Shukla & Shukla, 2017).

Moreover, using three or more antibiotics for 10 days was a risk factor for IPA development in COPD (Muquim et al. 2005; He et al. 2011). Another study found that four cases had used antibiotics in the last 3 months and a history of antibiotic use was the only risk factor for pulmonary fungal infection in one case that did not receive systemic steroid treatment (Agustí et al. 2003). In another study on 1209 patients with a positive respiratory culture for *Aspergillus* spp., Perfect et al. (2001) showed that COPD, corticosteroid use, and DM were the three main risk factors for fungal colonization.

## CONCLUSION

The prevalence of pulmonary fungal infection was significantly higher in COPD patients with comorbidities (77.8%) compared with COPD patients without comorbidities. There was predominance of *Candida* and *Aspergillus* spp., in both groups. Age, mechanical ventilation, corticosteroids therapy, and ICU admission were independent risk factors

associated with pulmonary fungal infection in COPD patients with comorbidities. COPD patients with comorbidities at risk for pulmonary fungal infection rate compared with COPD patients without comorbidities.

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